

09/993,777

-44-

includes the entire coding sequence of the ILTV gG gene (SEQ ID NO 7) and 733 of 986 amino acid codons from the 5' end of the g60 gene (SEQ ID NO 8). When this plasmid is used according to the DNA TRANSFECTION FOR GENERATING RECOMBINANT ILT VIRUS, it will replace the DNA coding
5 for the ILTV gG gene and amino acids 1 to 733 of the ILTV g60 gene with DNA coding for the *E. coli uidA* gene. A detailed description of the plasmid is given in Figures 9A-9D. It was constructed from the indicated DNA sources utilizing standard recombinant DNA techniques (42, 43). The plasmid vector pUC19 (Gibco, BRL) is derived from an approximately 2677 base pair *Asp*718I
10 to *Bam*HI fragment. Fragment 1 is an approximately 2830 base pair *Asp*718I to *Nhe*I subfragment of the ILTV 5164 bp *Asp*718I fragment (SEQ ID NO 1: Nucl. 1714-4544). Fragment 2 is an approximately 3051 base pair *Sal*I fragment containing the PRV gX promoter, *E. coli* β -glucuronidase (*uidA*) marker gene, and an HSV-1 TK polyadenylation site (See Figures 9A-9D).
15 Fragment 3 is an approximately 1709 base pair *Sal*I to *Bam*HI subfragment of the ILTV 4545 base pair *Bam*HI fragment (SEQ ID NO 1: Nucl. 7895-9604).

PLASMID 544-39.13. Plasmid 544-39.13 contains the β -glucuronidase expression cassette consisting of the PRV gX promoter, *E. coli* β -glucuronidase
20 (*uidA*) marker gene, and an HSV-1 TK polyadenylation site. A detailed description of the marker gene is given in Figures 10A-10D. It was constructed utilizing standard recombinant DNA techniques (42, 43) by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 10A-10D. The plasmid vector pSP71 (Promega) is derived
25 from an approximately 3066 base pair *Xma*I to *Sma*I fragment. Fragment 1 is an approximately 422 base pair *Sal*I to *Eco*RI restriction subfragment of the PRV *Bam*HI restriction fragment #10 (47). Note that the *Eco*RI site was introduced at the location indicated in Figures 12A-12D by PCR cloning. Fragment 2 is an approximately 1826 base pair *Eco*RI to *Sma*I fragment of the
30 plasmid pRAJ260 (Clonetech). Note that the *Eco*RI and *Xma*I sites were introduced at the locations indicated in Figures 10A-10D by PCR cloning. Fragment 3 is an approximately 784 base pair *Xma*I subfragment of the HSV-1